SSD: Sterol-Sensing Direct

Cholesterol homeostasis is established by a complex of three proteins, one of which contains a hydrophobic domain previously termed a sterol-sensing domain. New biochemical studies of this domain demonstrate direct high-affinity binding of the sterol-sensing domain to sterol.

In the July 23, 2004, issue of *Molecular Cell*, Goldstein and Brown have filled in another important piece of the picture of sterol metabolism (Radhakrishnan et al., 2004). The focus of recent work from this lab has been the intricate feedback mechanism that senses sterol, or its absence, and alters transcription of genes such as HMG CoA reductase and the LDL receptor, the activity of which leads to sterol synthesis and uptake, respectively (Brown and Goldstein, 1999). Previous work had shown that the input to the mechanism is a combination of cholesterol and also oxysterols, derivatives of cholesterol present at low levels. The mechanism consists of three proteins: (1) the transcription factor itself: sterol responsive element binding protein (SREBP), an integral membrane protein of the endoplasmic reticulum (ER) that is activated by cleavage to allow the transcriptionally active domain to enter the nucleus; (2) SREBP-cleavage activating protein (SCAP): a binding partner that mediates activation of SREBP by trafficking it to the Golgi, where cleavage occurs; and (3) INSIG: an integral membrane protein of the ER that anchors SCAP-SREBP complexes in the ER (Yang et al., 2002).

How might these three proteins respond to changes in the lipid content of the ER membrane? The most likely candidate for the lipid-sensitive switch in the SREBP-SCAP-INSIG mechanism was SCAP, which shares a region of sequence homology with several other proteins involved in cholesterol metabolism: HMG CoA reductase, patched, dispatched, 7-dehyrdocholesterol reductase, Neimann Pick type C protein 1 (NPC1), and NPC1-like 1 (NPC1L1). All these proteins are polytopic; i.e., they have domains made up of multiple (between 8 and 12) trans-membrane helices (TMHs). The region shared between these proteins consists of five TMHs and was originally described as being essential for the direct regulation of HMG CoA reductase by cholesterol, hence called a sterol-sensing domain (SSD) (Roitelman and Simoni, 1992).

Investigations of the way in which SCAP might respond to cholesterol started with the isolation of SCAP mutants that did not respond to sterol, and took a big step forward when a sterol-dependent switch was tentatively identified: a region of SCAP close to the SSD showed an altered pattern of tryptic digestion depending on whether cholesterol was present or not, and was independent of oxysterols (Brown et al., 2002). The altered digestion was inferred to mean that cholesterol might bind directly to the SSD and that a conformational change would ensue, leading to tighter SCAP-INSIG binding, thus retaining SREBP in the ER in the presence

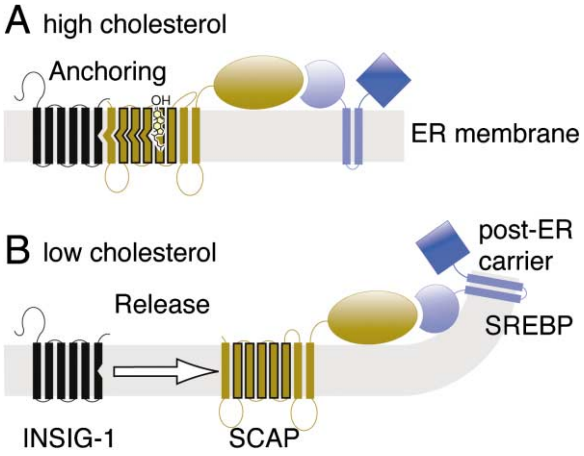

Figure 1. Cholesterol Binds Directly to SCAP, Inducing Its Retention in the Endoplasmic Reticulum

(A) SCAP (brown) binds cholesterol (yellow, not drawn to scale) and adopts a conformation that binds to INSIG (black), so becoming anchored in the ER.

(B) In the absence of cholesterol, SCAP is released from INSIG and enters the exocytic pathway along with SREBP (blue), which is cleaved in the Golgi to release the transcriptionally active domain (dark blue) into the cytoplasm (figure adapted from Loewen and Levine, 2002).

of cholesterol. This view was supported by work showing that binding to INSIG stabilizes the effect of cholesterol on SCAP (Adams et al., 2003). However, other data from expression of mammalian SREBP, SCAP, and INSIG in flies indicated that the mechanism might be indirect via more general biophysical effects on the ER membrane, because phosphatidylethanolamine, which is structurally unrelated to sterols, had a partial effect on SCAP retention (Dobrosotskaya et al., 2003).

In their new paper, Brown, Goldstein, and coworkers resolve this issue (Radhakrishnan et al., 2004). Simply put, they have purified the polytopic region of SCAP to homogeneity and shown a specific, direct interaction in vitro with cholesterol (half-maximal binding at 100 nM), which induces retention of SCAP in the ER (Figure 1). Binding was also shown for other compounds known to enhance the SCAP-INSIG interaction, including the aromatic neuroleptic drugs trifluoperazine and chlorpromazine, as well as a limited range of sterols including the plant lipid sitosterol. This brief description does little justice to the evident difficulties of working with a hydrophobic polypeptide binding a hydrophobic ligand in aqueous solution: just finding the right concentration of a detergent suitable for both protein and lipid was highly complex. The analysis of the peptide-lipid complex included size-exclusion chromatography, after which lipid still coeluted with peptide. As a by-product of this experiment, and verified in others, SCAP was found to form tetramers, the significance of which remains to be established.

Another piece of the jigsaw still not in place is the role of oxysterols. Highly bioactive compounds such as 25-hydroxycholesterol do not interact with SCAP in vitro (Radhakrishnan et al., 2004) and yet are essential for the response to sterol in vivo (Brown and Goldstein, 1999; Yang et al., 2002). One possibility is that oxysterols

mediate a signal from the plasma membrane and/or *trans*-Golgi network, where cholesterol is most abundant, back to the ER, which is sterol-poor, by inducing retrograde transport of excess cholesterol (Lange et al., 1999). A plausible mechanism involves a high-affinity receptor for 25-hydroxycholesterol, oxysterol binding protein (OSBP), and its homologs. It is now known that a large number of OSBP homologs are potential peripheral membrane proteins of the ER (Wyles and Ridgway, 2004), where they might influence the retrograde traffic of cholesterol that in turn may bind SCAP. It will be interesting to test the role of these proteins as putative regulators of the mechanism now described in so much detail.

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Selected Reading

Adams, C.M., Goldstein, J.L., and Brown, M.S. (2003). Proc. Natl. Acad. Sci. USA *100*, 10647–10652.

Brown, A.J., Sun, L., Feramisco, J.D., Brown, M.S., and Goldstein, J.L. (2002). Mol. Cell *10*, 237–245.

Brown, M.S., and Goldstein, J.L. (1999). Proc. Natl. Acad. Sci. USA *96*, 11041–11048.

Dobrosotskaya, I.Y., Goldstein, J.L., Brown, M.S., and Rawson, R.B. (2003). J. Biol. Chem. *278*, 35837–35843.

Lange, Y., Ye, J., Rigney, M., and Steck, T.L. (1999). J. Lipid Res. *40*, 2264–2270.

Loewen, C.J., and Levine, T.P. (2002). Curr. Biol. *12*, R779–R781.

Radhakrishnan, A., Sun, L.-P., Kwon, H.J., Brown, M.S., and Goldstein, J.L. (2004). Mol. Cell *15*, 259–268.

Roitelman, J., and Simoni, R.D. (1992). J. Biol. Chem. *267*, 25264–25273.

Wyles, J.P., and Ridgway, N.D. (2004). Exp. Cell Res. *297*, 533–547.

Yang, T., Espenshade, P.J., Wright, M.E., Yabe, D., Gong, Y., Aebersold, R., Goldstein, J.L., and Brown, M.S. (2002). Cell *110*, 489–500.